

Anti-Dentin Matrix Protein 1 (DMP1) Peptide antibody

Takara newly released a peptide antibody against Dentin Matrix Protein 1 (DMP1). DMP1, which had been studied as a protein specific to tooth dentin, was revealed to be useful as a new marker of bone cells. This peptide antibody is intended for studying localization of DMP1 in tissues and for performing western blot assay in bone extract.

About Dentin Matrix Protein 1 (DMP1)¹⁻⁵⁾

Dentin Matrix Protein 1 (DMP1) is a secretory acidic phosphoprotein that was identified from a cDNA library of tooth dentin. The gene is located in 4q21 on the human chromosome and forms a gene family with adjacent genes such as osteopontin, MEPE (matrix extracellular phosphoglycoprotein) and bone sialoprotein.

Recently, DMP1 has attracted attention for its expression in bone tissues. It is reported, from *in situ* hybridization and immunohistological staining of DMP1, that DMP is a bone matrix that is not produced in osteoblasts but specifically produced in bone cells buried in bone matrices.

The number of bone cells present in adult bone is estimated to be about 25,000 per cubic millimeter, which is ten times that of osteoblasts on bone surface. Bone cells are considered to play an important role in bone metabolism, although studies about bone cells are not fully advanced due to a lack of specific markers. However, antibodies against DMP1 are effective in bone cell studies since DMP1 can be used as a specific marker of bone cells.

Also, it is thought that DMP1 is closely related to calcification because it is negatively charged in tissues due to its amino acid sequence and it binds to calcium ions. In fact, facilitation of calcification was observed in a gene experiment in which DMP1 was introduced to an osteoblast cell line, revealing the possibility of using DMP1 as an important molecule for bone calcification studies.

About this product

[Preparation of peptide antibody]

Selection of a synthesized amino acid epitope

In reference to a paper by Dr. (Assistant Professor) Satoru Toyosawa³⁾ (Department of Oral Pathology, Graduate School of Dentistry, Osaka University), amino acid sequence position numbers 90 to 111 in rat DMP1: SGDDTFGDEDNGPGPEERQWGG was selected as an epitope. For immunization, a cysteine residue for cross-linking was introduced to the N terminal of the peptide. 10 mg of the 23-amino acid peptide was synthesized with 80% purity.

Preparation of antibody and affinity purification

2 mg of the above-mentioned peptide were cross-linked with maleimidated KLH and subcutaneously administered to a Japanese white rabbit, 4 times at intervals of 2 weeks with Freund's complete adjuvant, to obtain antiserum with 10,000-fold potency against the peptide. IgG fraction was obtained with a protein A column and the antigen-specific antibody was then highly purified with an affinity column on which 5 mg of peptide was immobilized.

[Product description]

- Amount of antibody
0.1 mg/vial (Carrier antibody not included. Specific antibody only)
Lyophilized product (Dissolved in PBS containing 1% BSA (without antiseptic) and lyophilized)
- Reconstitution of antibody
Dissolve lyophilized product in 50 ml of pure water to obtain 2 mg/ml concentration.
- Standard concentration for use 2 - 5 µg/ml
PBS containing 1% BSA or 25% BlockAce™ is recommended as dilution solution.

[Cross-reactivity]

Results of homology searches (Fig. 1) indicate that rat DMP1 amino acid sequence position numbers 90 to 111 show considerable homology with that of mouse and human. In fact, the reactivities to DMP1 in these animals are also confirmed by histological staining results.



Figure 1. Peptide alignment of DMP1

Western blot assay

Toyosawa *et al.*³⁾ confirms that a peptide antibody that has anti-DMP1 (90 - 111) as an epitope, reacts with rat DMP1 protein expressed in *Escherichia coli* and naturally occurring DMP1 protein in rat bone extract.

This article provides an example in which western blot assay was carried out using this peptide antibody for rat skull EDTA extract.

[Methods]

Preparation of bone extract

1. Connective tissues were removed as carefully as possible from 70 skulls of 24-day-old SD rats (female) and the skulls were collected after washing with PBS (20 g yield).
2. After pounding with pestle in about 1 liter of liquid nitrogen, 100 ml of extraction solution containing 0.5 M EDTA 2Na was immediately added to perform extraction at 4°C for 72 hours.

[Composition of extraction solution]

0.5 M EDTA 2Na
5 mM benzamidine
30 mM PMSF
10 mM aminocaproic acid
1 mg/ml leupeptine
20 mM Tris-HCl (pH 7.8)

3. The extract was centrifuged (9,000 rpm) and the supernatant was dialyzed against 5 l of pure water a total of 4 times over 2 days.
4. The dialyzed solution (about 100 ml) was stored at -80°C and an aliquot was taken as a sample for western blot assay.

Western blotting

Rabbit serum before immunization (control), anti-DMP1 peptide antiserum before purification, protein A purified antibody, affinity purified antibody (this product) and rabbit purified IgG (control) were used to compare the results.

1. After samples were reduced and heated, 10 ml of each were applied to each lane of 5 - 20% SDS gradient gel and SDS-PAGE was performed according to a common procedure.
2. Protein on the gel was transferred to PVDF membrane after electrophoresis.
3. After blocking with BlockAce™ (stock solution), the PVDF membrane was cut along each lane, drenched in the solutions listed below and reacted for an hour at room temperature.

- A: Rabbit serum before immunization (1,000-fold dilution)
- B: Anti-DMP1 peptide antiserum before purification (1,000-fold dilution)
- C: Protein A purified antibody (10 µg/ml)
- D: Affinity purified antibody (10 µg/ml)
- E: Rabbit purified IgG (10 µg/ml)

4. After washing and shaking with PBS containing 0.1% Tween, the membrane was drenched in 500-fold dilution of anti-rabbit IgG-POD labeled antibody (ICN) and reacted for an hour at room temperature. After washing and shaking with PBS containing 0.1 % Tween again, the membrane was colored with coloring substrate TrueBlue™.

[Results]

When antiserum before purification and purified antibody were used, a clear band was observed in the vicinity of a molecular weight of 23,000 (Fig. 2). However, the detected band was much smaller than its theoretical size, probably because the process of bone extract preparation degraded DMP1 protein.

It was confirmed that the antibody specificity increased as purification proceeded. Some bands which appeared to be nonspecific were observed in the region of low molecular weight with protein A purified antibody, while a single band was seen with affinity purified antibody (this product).

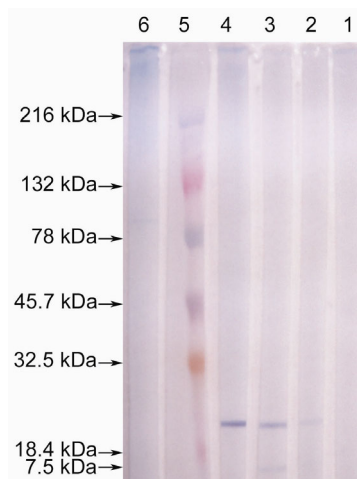


Figure 2. Western blotting

Lane

- 1 : Rabbit serum before immunization (× 1,000)
- 2 : Anti-DMP1 peptide antiserum before purification (× 1,000)
- 3 : Protein A purified antibody (10 µg/ml)
- 4 : Affinity purified antibody (10 µg/ml)
- 5 : Molecular weight marker
- 6 : Control rabbit purified IgG (10 µg/ml)

Immunohistological staining

An example of an immunostaining experiment using this product is given below. This experiment was conducted by Dr. Toyosawa's research group, referred to above.

[Methods]

Preparation of tissue section

Material: Bone tissue from 3-week-old rats

Procedure:

1. Immobilization with 4% paraformaldehyde solution
2. Decalcification with 10% EDTA (at 4°C, for 1 week)
3. Alcohol dehydration
4. Xylene clearing
5. Paraffin embedding
6. Slicing
7. Immunostaining

Immunohistological staining

Primary antibody: Rabbit anti-rat DMP1 (90-111) Polyclonal antibody (1,000-fold dilution)

Tissue section: Rat EDTA decalcified paraffin embedded section

Detection method: sABC system (DAKO A/S)

Staining procedure:

1. Deparaffination
2. Activation of antigen: Treated with trypsin solution at 37°C for 20 min

[Composition of Trypsin solution]

Trypsin 25 mg

Calcium chloride 25 mg

0.01 M phosphate buffered saline (PBS) 100 ml

3. Blocking of nonspecific protein
4. Primary antibody, at 4°C, over a night
5. Biotin-labeled secondary antibody at room temperature for 30 min
6. Blocking of endogenous peroxidase
7. Reaction of Strept ABC complex at room temperature for 30 min
8. DAB reaction
9. Counterstaining (methylgreen)
10. Dehydration, clearing, embedding

[Results]

Results of immunostaining of rat bone tissue using this product are shown in Fig. 3.

Specifically strong positive response was observed in matrices around bone cells including bone canaliculi in jaw bone and cervical spine cortical bone, while no response was found around trabecular bones that correspond to osteoids.

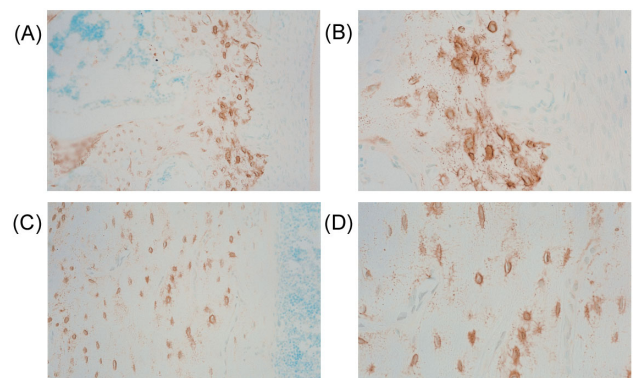


Figure 3. Examples of immunohistological staining using this product

(A): Rat jaw bone (× 66)

In jaw bone, specifically strong positive response is observed in matrices around bone cells including bone canaliculi. Immunological response is not found around trabecular bones that correspond to osteoids.

(B): High-power field of A (×132)

(C): Cortical bone of rat cervical spine (× 66)

In cervical spine cortical bone, specifically strong positive response is observed in matrices around bone cells including bone canaliculi. Immunological response is not found around trabecular bone that corresponds to osteoids.

(D): High-power field of C (×132)

[References]

George, A. *et al.* (1993) *J. Biol. Chem.*, **268**, 12624-12630.

Toyosawa, S. *et al.* (2000) *J. Mol. Evol.*, **50**, 31-38

Toyosawa, S. *et al.* (2001) *J. Bone Miner. Res.*, **16**, 2017-26

Narayanan, K. *et al.* (2001) *Proc. Natl. Acad. Sci. USA*, **98**, 4516-4521

Toyosawa, S. *et al.* (2002) *Tissue Engineering for Therapeutic Use* **6**, 83-94